Flexor tendon grafts for pulley reconstruction - Morphological aspects

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Abstract: BACKGROUND: Pulleys are crucial to convert flexor tendon excursion into angular motion at the metacarpophalangeal and interphalangeal joints. Loss of pulley function can lead to significant impairment of hand function and may require surgical reconstruction. This reconstruction can be achieved using different flexor tendons grafts, such as the intrasynovial flexor digitorum superficialis (FDS) or the extrasynovial palmaris longus (PL). However, there is limited knowledge on the micromorphology of human pulleys and the suitability of flexor tendon grafts for their reconstruction remains elusive. METHODS: In the present cadaver study A2 and A4 pulleys were compared with FDS and PL tendons by means of scanning electron microscopy (SEM), histology and immunohistochemistry. Surface morphology, core structure and vascularization of the specimens were analyzed. RESULTS: SEM imaging of the gliding surfaces revealed morphological differences between tendons and pulleys. Moreover, the core structure of FDS samples was characterized by bundles of individual collagen fibrils whereas PL tendons exhibited a less hierarchical microstructure. In contrast, pulleys consisted of lamellar sheets of densely packed collagen fibrils. Finally, immunohistochemical analyses revealed that the flexor tendons and pulleys contain similar numbers of $CD31^{+}$ microvessels, indicating a comparable tissue vascularization. CONCLUSION: This study provides novel SEM and immunohistochemical insights into the micromorphology of human pulleys and flexor tendon grafts. Intrasynovial flexor tendons may be particularly suitable for pulley reconstruction and preserving the paratenon may be crucial for graft revascularization.

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RESEARCH ARTICLE

Flexor tendon grafts for pulley reconstruction – Morphological aspects

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ABSTRACT

Background: Pulleys are crucial to convert flexor tendon excursion into angular motion at the metacarpophalangeal and interphalangeal joints. Loss of pulley function can lead to significant impairment of hand function and may require surgical reconstruction. This reconstruction can be achieved using different flexor tendons grafts, such as the intrasynovial flexor digitorum superficialis (FDS) or the extrasynovial palmaris longus (PL). However, there is limited knowledge on the micromorphology of human pulleys and the suitability of flexor tendon grafts for their reconstruction remains elusive.
Methods: In the present cadaver study A2 and A4 pulleys were compared with FDS and PL tendons by means of scanning electron microscopy (SEM), histology and immunohistochemistry. Surface morphology, core structure and vascularization of the specimens were analyzed.
Results: SEM imaging of the gliding surfaces revealed morphological differences between tendons and pulleys. Moreover, the core structure of FDS samples was characterized by bundles of individual collagen fibrils whereas PL tendons exhibited a less hierarchical microstructure. In contrast, pulleys consisted of lamellar sheets of densely packed collagen fibrils. Finally, immunohistochemical analyses revealed that the flexor tendons and pulleys contain similar numbers of $CD31^+$ microvessels, indicating a comparable tissue vascularization.
Conclusion: This study provides novel SEM and immunohistochemical insights into the micromorphology of human pulleys and flexor tendon grafts. Intrasynovial flexor tendons may be particularly suitable for pulley reconstruction and preserving the paratenon may be crucial for graft revascularization.

1. Introduction

Pulleys are fibrous tissue condensations, keeping the flexor tendons adjacent to the hand skeleton (Zafonte et al., 2014). They are crucial to convert tendon excursion into angular motion at the metacarpophalangeal and interphalangeal joints, allowing full finger flexion (Mehta and Phillips, 2005). In the human hand, the pulley system of the long fingers consists of the annular A1–A5 pulleys and the cruciate C1–C3 pulleys. The A2 and A4 pulleys insert to the shaft of the proximal and middle phalanx and are key to prevent bowstringing of the flexor tendons (Doyle, 1989; Tomaino et al., 1998). Pulley insufficiency after trauma or pyogenic flexor tenosynovitis can lead to significant impairment of hand function, often characterized by loss of flexion and strength (Langer et al., 2015; Marco et al., 1998; Wiater et al., 2013). Traumatic pulley injury is frequently observed in rock climbers and while partial injuries respond well to conservative treatment, complex insufficiency including multiple pulleys commonly requires surgical reconstruction (Schneeberger and Schweizer, 2016; Schöffl et al., 2003).

Pulley reconstruction can be achieved using different autologous grafts, such as the flexor digitorum superficialis (FDS) and palmaris longus (PL) tendons or extensor retinaculum (Arora et al., 2007; Clark et al., 2010). While numerous studies evaluated different techniques of pulley reconstruction, there is limited knowledge on the morphological characteristics of human pulleys and on the mechanical suitability of the above-mentioned tendon grafts for

Abbreviations: FDS, flexor digitorum superficialis; PL, palmaris longus; SEM, scanning electron microscopy.

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pulley reconstruction. Based on the scarce literature on the morphology of human pulleys, we hypothesized that there are relevant differences in the collagen microstructure and vascularization of flexor tendons and pulleys. The present cadaver study was designed to compare A2 and A4 pulleys with PL and FDS tendon grafts by means of scanning electron microscopy (SEM), histology and immunohistochemistry. For this purpose, surface morphology, core structure and vascularization of the specimens were analyzed.

2. Materials and methods

2.1. Cadaver dissection

FDS ($n = 8$) and PL ($n = 4$) tendons as well as A2 and A4 pulleys ($n = 8$) were harvested from 8 fingers of 4 fresh-frozen human hands. Two hand surgeons dissected the complete flexor tendon/pulley apparatus (Fig. 1). Individual FDS slings, PL tendons and A2/A4 pulleys were harvested and the corresponding synovial surfaces of FDS and the pulleys were marked with a monofilament suture to allow orientation for the subsequent SEM imaging. For histological analyses, the visceral synovial sheath (i.e. epitenon – Cohen and Kaplan, 1987) around the FDS tendons as well as the paratenon of the PL tendons was preserved (in the following referred to as *paratenon* for both tendons). The cadaver hands were from male body donors of the body donation program of the Saarland University. Mean donor age was 66 ± 8 y and no diagnoses affecting hand function were recorded. The bodies were frozen within a post mortem interval of 24 h and thawed before dissection. All donors gave informed consent during lifetime to all procedures necessary for teaching and research.

2.2. Scanning electron microscopy

To assess the microstructure of excised pulleys and flexor tendons by means of SEM, they were rinsed in phosphate buffered saline (PBS) to remove loosely attached tissue and blood cells from the outer surfaces. The samples were fixed in 2 vol% glutaraldehyde (Science Services GmbH, Munich, Germany) in 0.1 M sodiumcacodylate buffer at pH 7.4 (Carl Roth GmbH & Co KG, Karlsruhe, Germany) for 2 h at room temperature under slight movement followed by storage at 4 °C for 48 h. Subsequently, the samples were rinsed in 0.1 M sodiumcacodylate buffer and were then incubated in 1 vol% osmium tetroxide in 0.2 M sodiumcacodylate buffer for 2 h under slight movement at room temperature in the dark. Osmium tetroxide was removed by four incubations in deionized water (dH2O) for 20 min each. Prior to sputtering, the samples had to be dehydrated carefully in an ascending ethanol series (70 vol%, 80 vol%, 90 vol%, 96 vol%, 100 vol%) under slight movement for 30 min each, followed by incubation in a mixture (50:50) of 100 vol% ethanol and hexamethyldisilazane (15 min, Carl Roth GmbH Co KG) and in pure hexamethyldisilazane (15 min). The samples were then covered with pure hexamethyldisilazane and placed under the laboratory hood over night. The completely dehydrated samples were then transferred to conductive carbon adhesive tabs (Plano GmbH, Wetzlar, Germany), and were sputtered with gold 3 × for 60 s (SCD 005, Balzers Union, Balzers, Liechtenstein). A FEI XL 30 ESEM FEG SEM device (FEI, Hillsboro, OR, USA) was used under high vacuum conditions at an acceleration voltage of 5 kV in secondary electrones-mode for a qualitative analysis of the ultrastructure.

2.3. Histology and immunohistochemistry

Formalin-fixed samples were embedded in paraffin and cut into 3 μm-thick sections. Sections were stained with hematoxylin and eosin (HE). Additional sections were stained with a monoclonal rabbit anti-human antibody against CD31 (1:100; Abcam, Cambridge, UK). A goat anti-rabbit IgG-Alexa555 antibody (1:100; Thermo Fisher Scientific Inc., Waltham, MA, USA) served as secondary antibody. Cell nuclei were stained with Hoechst 33342

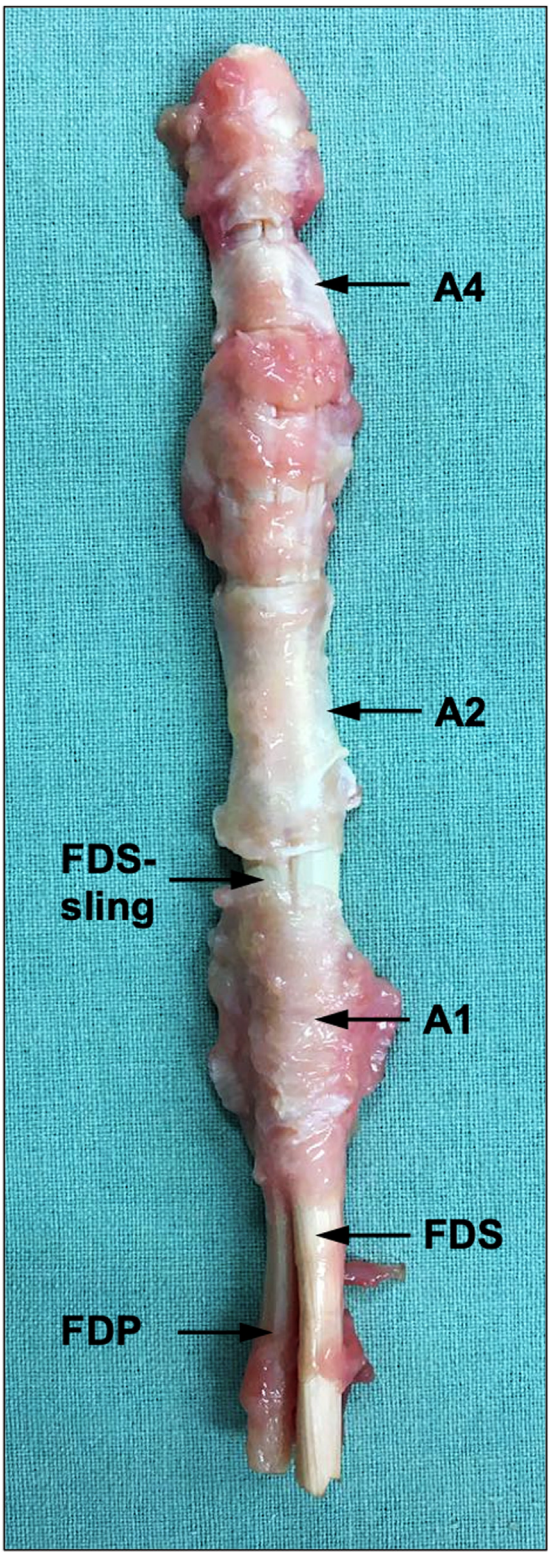

Fig. 1. Specimen dissection. Fotograph illustrating the harvested flexor tendon/pulley apparatus from the palm to the tip of the finger. FDS = flexor digitorum superficialis, FDP = flexor digitorum profundus.

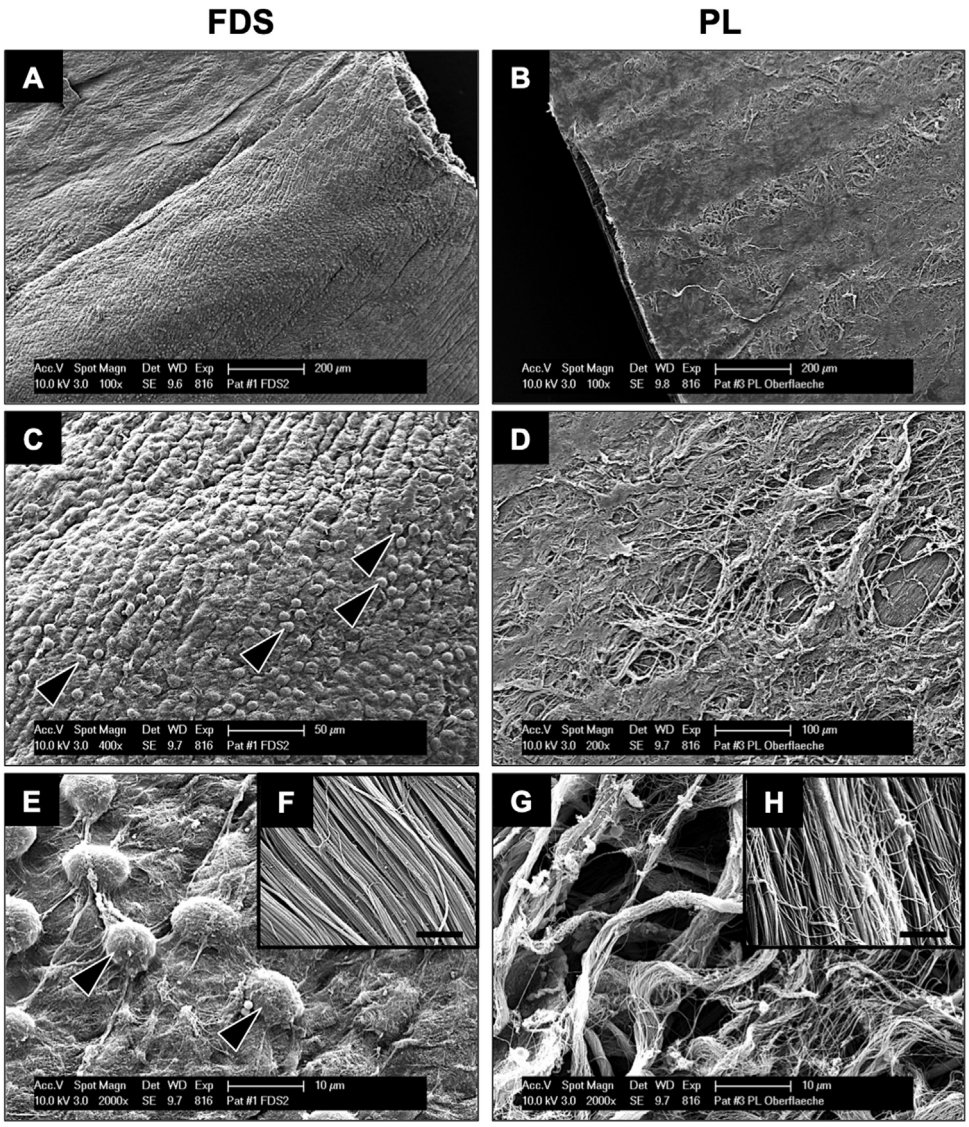

Fig. 2. Surface morphology of FDS and PL tendons. SEM images illustrating the surface of FDS (A, C, E, F) and PL (B, D, G, H). The synovial layer of FDS tendons is characterized by a dense network of synovial cells (arrowheads in C and E). In contrast, the extrasynovial PL exhibits a different surface morphology (D and G). Characterization of individual collagen fibrils of FDS (F) and PL (H). Scale bars: F and H = 2 µm. FDS = flexor digitorum superficialis, PL = palmaris longus, SEM = scanning electron microscopy.

(2 µg/mL; Sigma-Aldrich, Taufkirchen, Germany). The density of $CD31^+$ microvessels (given in mm^{-2}) was assessed within 4 regions of interest of each sample using a BX60 microscope (Olympus, Hamburg, Germany). Microvessel density was analyzed in the paratenon of FDS and PL tendons as well as in the superficial layer of A2 and A4 pulleys (i.e., the superficial areolar layer opposite to the flexor tendon).

2.4. Statistics

Data were analyzed for normal distribution and equal variance. One-way analysis of variance (ANOVA) was used to compare groups, followed by the Tukey's post hoc test. Data are shown in box plots, where the horizontal line represents the median and the plus represents the mean. Statistical significance was accepted for $P < 0.05$. The statistical analysis was performed using Prism 8 (GraphPad Software, Inc., San Diego, CA, USA).

3. Results

3.1. Surface morphology and collagen network of flexor tendons

SEM imaging revealed that the synovial surface of FDS tendons is characterized by a dense layer of synovial cells (Fig. 2A, C and E). In contrast, the extrasynovial PL tendon exhibits a different surface morphology with an amorphous layer (Fig. 2B and D). Underneath the synovial membrane, the longitudinal orientation of individual collagen fibrils in FDS tendons is highly consistent whereas PL tendons show a different and less homogenous fibrillar architecture (Fig. 2F and H).

3.2. Surface morphology and collagen network of pulleys

The gliding surface of A2 and A4 pulleys is characterized by an amorphous layer (Fig. 3A and B). In contrast to flexor tendons, the underlying collagen fibrils of pulleys are interwoven and organized

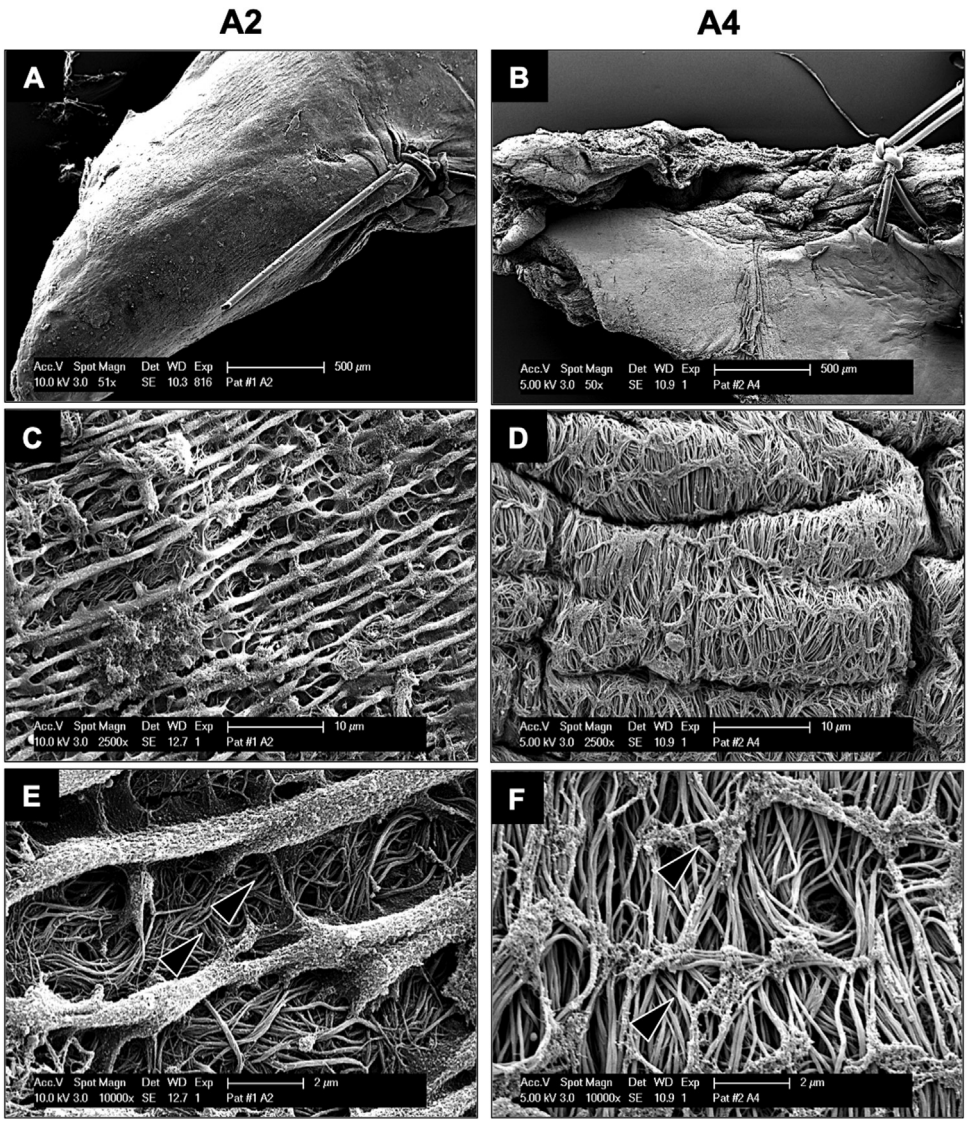

Fig. 3. Surface morphology of A2 and A4 pulleys. SEM images of the gliding surface of A2 (A) and A4 (B) pulleys. Compared with flexor tendons, the collagen fibrils underneath the synovial membrane (C–F) are characterized by a different structure. As indicated in E and F, individual fibrils (arrowheads) are not aligned strictly parallel but rather interwoven and in a mesh-like formation. SEM = scanning electron microscopy.

in a mesh-like formation (Fig. 3C–F). While the fibrillar architecture appears to be random at 10,000× magnification (Fig. 3E and F), lower magnification reveals ridges and furrows perpendicular to the digital axis (Fig. 3D).

3.3. Core structure of pulleys and flexor tendons

Further SEM imaging of transversally cut pulleys and tendons was performed to investigate the morphology and orientation of the core structure. In pulleys, individual collagen fibrils are assembled to sheet-like layers. Numerous of these sheets are aligned to form the tight annular structure of a pulley (Fig. 4A and B). Interestingly, the core structure of flexor tendons is significantly different. In fact, FDS tendons are characterized by bundles of circularly oriented collagen fibrils (Fig. 4C and D) whereas the core structure of PL tendons is less hierarchical (Fig. 4E and F). The density of collagen fibrils is higher in the core structure of pulleys when compared to FDS and PL samples (Fig. 4B, D and F).

3.4. Histological and immunohistochemical analysis of FDS, PL and pulleys

The qualitative evaluation of HE-stained sections confirmed the structural differences between FDS and PL tendons with a markedly lower density of collagen fibrils in the latter (Fig. 5A and C). The histological structure of A2 and A4 pulleys is different as indicated in Fig. 5E and G. In fact, it is characterized by a dense eosinophilic core structure and an areolar outer layer (i.e., the opposite layer to the gliding surface), containing the nourishing microvasculature.

Finally, immunohistochemical staining was performed to assess the microvascular network of flexor tendons and pulleys (Fig. 5B, D, F and H). Because the collagenous center of all specimens exhibited scarce microvessels, quantification was performed in the well-vascularized paratenon of FDS and PL and in the outer areolar layer of A2 and A4 pulleys. Mean microvessel density was $100 \pm 52\ mm^{-2}$ for FDS and $68 \pm 33\ mm^{-2}$ for PL tendons as well as $105 \pm 60\ mm^{-2}$

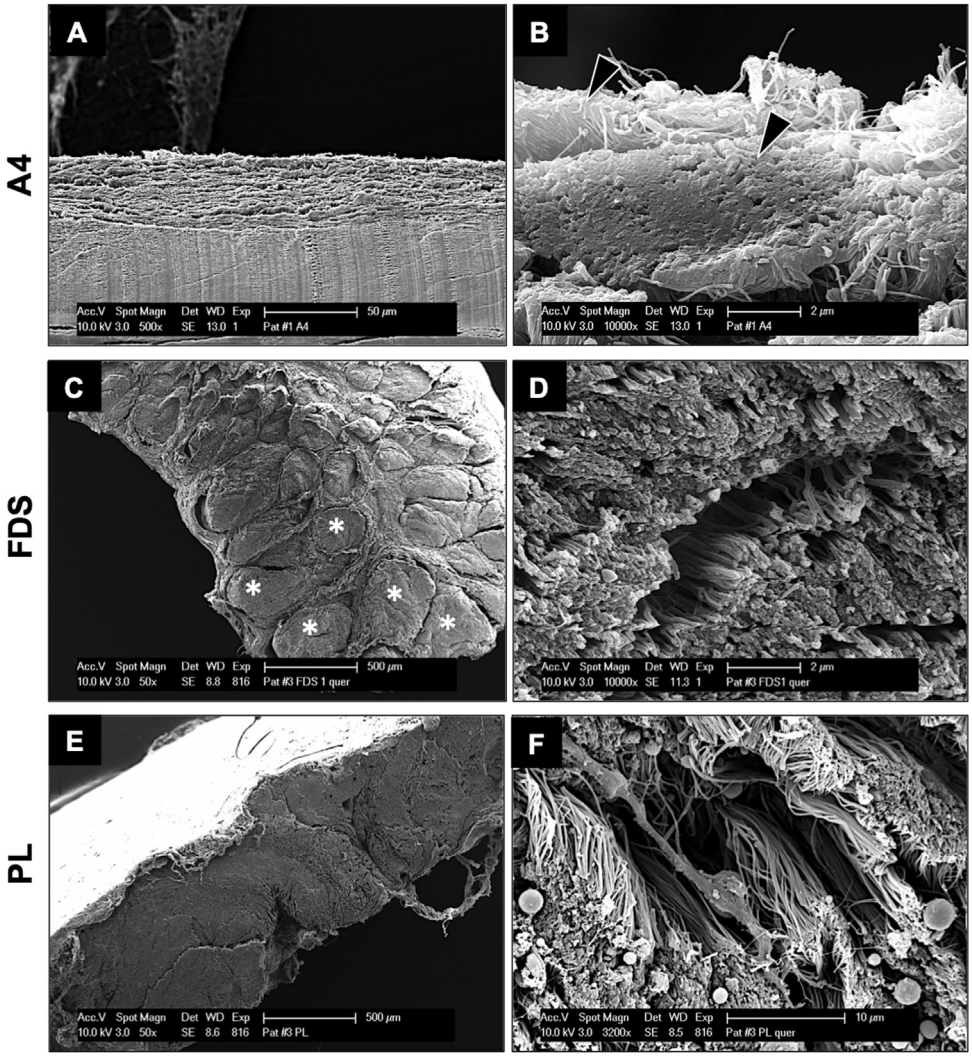

Fig. 4. Core structure of A4 and flexor tendons. SEM images of an A4 pulley (A and B) and FDS (C and D) as well as PL (E and F) tendons. The collagen fibrils of the pulley are aligned to lamellar sheets, resulting in a carpet-like architecture (A and B). In contrast, FDS tendons are characterized by bundles (asterisks in C) of collagen fibrils. The collagen network of PL tendons (E and F) exerts a lower level of organization. In the pulley, collagen fibrils appear to be packed more densely (B, arrowheads) when compared to the microstructure of FDS (D) and PL (F) tendons. FDS = flexor digitorum superficialis, PL = palmaris longus, SEM = scanning electron microscopy.

for A2 and $78 \pm 30\ mm^{-2}$ for A4 pulleys, respectively (Fig. 5I). There was no significant difference between the four groups, indicating a comparable vascularization of flexor tendons and pulleys.

4. Discussion

Pulley reconstruction is a rare and challenging procedure in hand surgery and often the results are biomechanically not satisfactory. In the last decades, only few authors investigated the microstructure of human pulleys and, therefore, specific requirements for grafts used in pulley reconstruction remain elusive. In the present cadaver study, we found significant morphological differences of the collagen network of flexor tendons and pulleys. Our investigation confirms the findings of previous SEM imaging of the human A1 pulley (Sbernardori et al., 2007). In fact, ridges and furrows perpendicular to the digital axes characterize the gliding surface of normal A1 pulleys. Our results reveal that the A2 and A4 pulleys exhibit a comparable surface morphology. Moreover, SEM imaging of the underlying collagen network indicates a highly organized lamellar architecture of individual fibrils. In contrast, FDS and PL tendons exhibit a different fibrillar architecture. Particularly PL tendons are characterized by a lower density of collagen fibrils and it may be assumed that this structural difference is associated with less favorable biomechanics for pulley reconstruction. The different fibrillar architecture of FDS and PL tendons is probably due to different loading patterns of the tendons in vivo and may rapidly change after grafting. Nevertheless, the herein presented SEM imaging of FDS and PL tendons further contributes to understand the value of intrasynovial and extrasynovial tendon grafts for pulley reconstruction.

Beside micromorphology, biomechanical characteristics such as tensile strength or frictional force are critical for pulley reconstruction (Lin et al., 1990; Mallo et al., 2008). Interestingly, experiments with canine and human specimens revealed that intrasynovial tendon grafts are associated with significantly less frictional forces and excursion resistance when compared to extrasynovial grafts (Nishida et al., 1998; Nishida et al., 1999; Uchiyama et al., 1997). Furthermore, it was demonstrated that pulley reconstruction using intrasynovial flexor tendons is also associated with significantly less frictional resistance when compared to extrasynovial grafts (Seiler et al., 1998). Finally, the biomechanical role of the paratenon of extrasynovial tendon grafts was investigated by the same research group (Momose et al., 2002). They found that preserving the paratenon of human PL tendons significantly

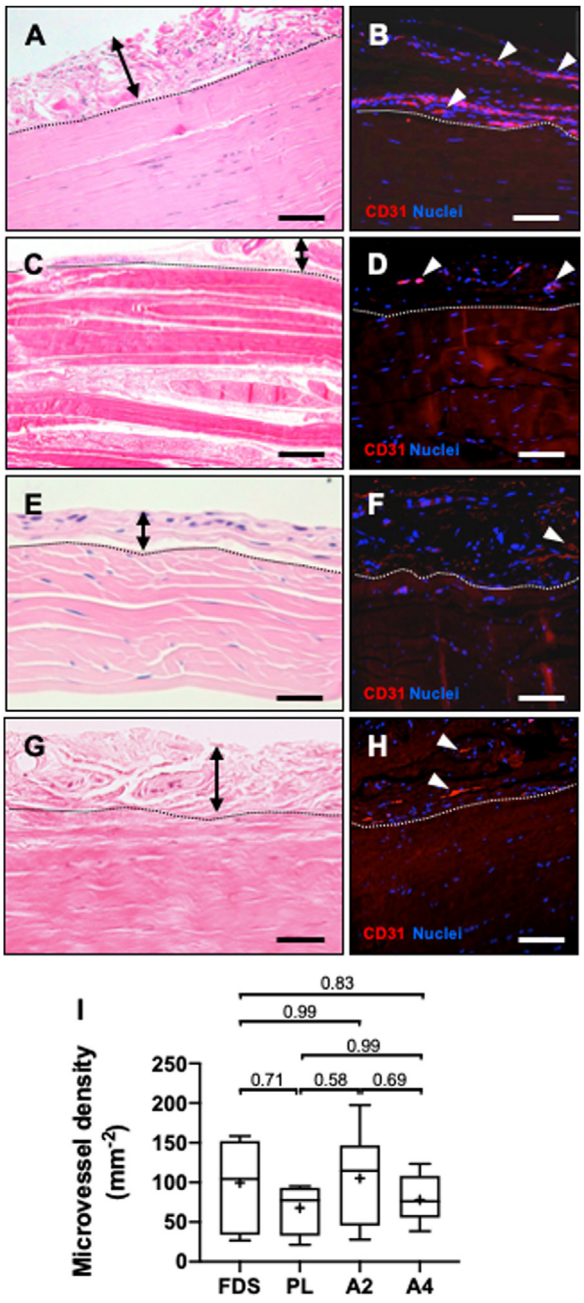

Fig. 5. Histology/immunohistochemistry of FDS, PL and pulleys. HE- (A, C, E and G) and CD31-stained sections (B, D, F and H) of FDS (A and B) and PL (C and D) tendons as well as of A2 (E and F) and A4 (G and H) pulleys. Microvessel analysis was performed in the paratenon of FDS and PL as well as the areolar layer of A2 and A4 (double arrows in A, C, E and G; dashed lines in A–H = border between analyzed layer and core structure). White arrowheads = individual microvessels. Quantification of microvessel density (I). $n = 7–8$ for FDS, A2 and A4 and $n = 4$ for PL. Scale bars: A = 150 μm; B, D, F and H = 100 μm; C = 200 μm; E and G = 25 μm. FDS = flexor digitorum superficialis, HE = hematoxylin and eosin, PL = palmaris longus.

decreases the gliding resistance when compared to tendons without paratenon.

Angiogenesis is essential for the remodeling of autologous tendon grafts (Petersen et al., 2003; Pufe et al., 2005). In a sheep model, immunohistochemical analyses revealed that six weeks after transplantation for ligament reconstruction, Achilles tendons were surrounded by a highly vascularized reparative tissue expressing the angiogenic peptide vascular endothelial growth factor (VEGF) (Petersen et al., 2003). Of note, graft revascularization is driven by microvessel ingrowth from the synovial envelope toward the center of transplanted tendons (Unterhauser et al., 2003). In our study, immunohistochemical analyses were performed to quantify the vascularization of human pulleys and flexor tendon grafts. The core structure of flexor tendons mainly consists of poorly vascularized collagen bundles, resulting in low counts of microvessels (Younesi et al., 2017). Accordingly, the quantification and comparison of tissue vascularization of pulleys and flexor tendons including the inner collagen layer was unreliable in our hands. Therefore, the microvessel density was only calculated in the well-vascularized outer areolar layer of pulleys and the paratenon of flexor tendons. Interestingly, the vascularization of pulleys and flexor tendons was not significantly different. These findings indicate that FDS and PL tendons exhibit a similar vascularization potential through inosculation, i.e. the connection of the graft's microvascular network to the microvessels of the surrounding hosting tissue after transplantation. Hence, from the clinician's perspective, preserving the paratenon of a tendon graft may not only reduce friction but also enhance revascularization.

The present study has important limitations. First, the number of specimens is low and the analysis of SEM imaging was qualitative. Second, the surface analysis of flexor tendons and pulleys is depending on pre-existing degenerative processes, such as trigger finger or tenosynovitis. For this purpose, we only investigated A2 and A4 pulleys and not the most frequently affected A1 pulley. Finally, our findings are based on a cadaver study, which complicates their translation into clinical practice. Taken together, we herein provide novel findings on the micromorphology and vascularization of human pulleys and flexor tendons that are of interest for hand surgeons dealing with pulley reconstruction. Intrasynovial flexor tendons may be particularly suitable for pulley reconstruction and preserving the paratenon may be crucial for graft revascularization.

Declarations of interest

None.

Authors' contribution

TF: Conceptualization; Methodology; Investigation; Data curation; Visualization; Roles/Writing – original draft; PD: Conceptualization; Methodology; Investigation; Roles/Writing – original draft; TP: Conceptualization; Methodology; Writing – review & editing; MC: Conceptualization; Methodology; Writing – review & editing; TS: Conceptualization; Methodology; Roles/Writing – original draft; MDM: Conceptualization; Methodology; Writing – review & editing; WM: Conceptualization; Methodology; Investigation; Roles/Writing – original draft; FSF: Conceptualization; Methodology; Investigation; Formal analysis; Visualization; Roles/Writing – original draft

Ethical statement

The cadaver specimens used in the present study were from body donors of the body donation program of the Saarland University, Germany. All donors gave informed consent during lifetime to all procedures necessary for teaching and research.

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